Phosphofructokinase (PFK) Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Catalog Number: NA0827

Size: 50T/48S

Components:

Extract Solution: Liquid 60 mL×1. Storage at 4℃.

Reagent I: Liquid 40 mL×1. Storage at 4℃.

Reagent II: Powder ×1. Storage at -20℃. Add 2.8 mL of distilled water before use and dissolve it fully.

Reagent III: Liquid45μL ×1. Storage at -20℃. The Liquid is placed in the EP tube in the reagent bottle. According to the volume ratio of Reagent III: distilled water is 2:13, mix well before use. prepared when the solution will be used; it is recommended to pack and store the unused Reagent III solution at -20℃ to avoid repeated freezing and thawing;

Reagent IV: Liquid 20μL ×1. Storage at 4℃. The liquid is placed in the EP tube in the reagent bottle. According to the volume ratio of Reagent III: distilled water is 4:65, mix well before use. prepared when the solution will be used; and the mixture is ready to use;

Preparation of PFK working fluid (25 samples can be measured): Take 19 mL of Reagent I and 1.26 mL of Reagent II and mix them thoroughly

Product Description

PFK (EC 2.7.1.11) is one of the key regulatory enzymes in the process of glycolysis, which widely found in animals, plants, microorganisms and cultured cells. It is responsible for converting fructose-6-phosphate and ATP into fructose-diphosphate and ADP.

PFK catalyzes the formation of fructose-1,6-diphosphate and ADP to from fructose-6-phosphate and ATP. Pyruvate kinase and lactate dehydrogenase further catalyze the oxidation of NADH to generate NAD^+. The degradation rate of NADH which measured at 340 nm is used to reflect the activity of PFK.

Reagents and Equipment Required but Not Provided.

Table centrifuge, water-bath, UV spectrophotometer, 1 mL quartz cuvette, adjustable pipette, mortar/homogenizer, ice and distilled water.

Procedure

1. Sample pretreatment

a. Bacteria or cells

Collect the bacteria or cells into a centrifuge tube, discard the supernatant after centrifugation. It is suggested that add 1 mL of Extract solution into per 5 million bacteria or cells, ultrasonic crushing bacteria or cells (power 200W, ultrasonic of 3s, interval of 10s, repeat 30 times); Centrifuge at 8000 ×g for 10

minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

b. Tissue:

It is suggested that add 1 mL of Extract solution to 0.1 g of tissue. Homogenate on ice bath. Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before test.

c. Serum (plasma) sample: Direct detection.

II. Determination

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 340nm, set zero with distilled water.

2. Determination procedure and sample table

Reagent Name (μL)	Test Tube (T)
PFK Working Solution	800
Sample	30
Reagent III	5
Reagent IV	5

Add the above reagents to 1 mL quartz cuvette in sequence and start timing when add samples. Detect the absorbance at 340 nm at the time of 20 seconds record as A1(20s). then place dishes with the reaction solution in a 37℃ (mammal) or 25℃ (other species) water bath for 10 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A2(620s). ΔA=A1-A2.

Note:

1. Place Reagent III, Reagent IV and samples on ice during the determination process to avoid denaturation and inactivation.
2. The temperature of the reaction solution in the cuvette must be maintained at 37℃ or 25℃. Take a small beaker and add in a certain amount of distilled water and must keep the temperature be at 37℃ or 25℃. Place the beaker in a 37℃ or 25℃ water bath. In the reaction process, place the cuvette with the reaction solution in the beaker.
3. It is better for two people to do this experiment at the same time, one for measure the absorbance and one for timing to ensure the accuracy of the experimental results.
4. Dilute the enzyme liquid with Extract solution if ΔA is greater than 0.5, making ΔA is less than 0.5 to improve the detection sensitivity.

III. Calculation of PFK activity unit:

a. Serum (plasma):

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the conversion of 1 nmol of fructose-6-phosphate and 1 nmol of ATP into 1 nmol of fructose-1, 6-diphosphate and 1 nmol of ADP per minute in the reaction system every milliliter of serum (plasma).

PFK (U/mL)=[$\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9$] $\div V_S \div T = 450 \times \Delta A$

b. Tissues, bacteria or cells:

（1）Calculated by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the conversion

of 1 nmol of fructose-6-phosphate and 1 nmol of ATP into 1 nmol of fructose-1, 6-diphosphate and 1 nmol of ADP per minute in the reaction system every milligram of tissue protein.

PFK (U/mg prot)=[$\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9$] ÷($W \times V_S \div V_{TS}$)÷T=450× ΔA ÷Cpr

（2）Calculated by sample fresh weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the conversion of 1 nmol of fructose-6-phosphate and 1 nmol of ATP into 1 nmol of fructose-1, 6-diphosphate and 1 nmol of ADP per minute in the reaction system every gram.

PFK (U/g fresh weight)=[$\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9$] ÷($W \times V_S \div V_{TS}$)÷T=450× ΔA ÷W

(3) Calculate by bacterial or cell density

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the conversion of 1 nmol of fructose-6-phosphate and 1 nmol of ATP into 1 nmol of fructose-1, 6-diphosphate and 1 nmol of ADP per minute in the reaction system every 10,000 bacteria or cells.

PFK (U/10^4 cell)=[$\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9$] ÷($500 \times V_S \div V_{TS}$)÷T=0.9× ΔA

V_{TV}: Total volume of the reaction system, 0.84×10^{-3} L;

ε: The molar extinction coefficient of NADPH, 6.22×10^3 L/mol/cm;

d: Light path of the cuvette, 1 cm;

V_S: Sample volume, 0.03 mL;

V_{TS}: Extract solution volume, 1 mL;

T: Reaction time, 10 minutes;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

10^9: Unit conversion factor, 1 mol=10^9 nmol;

500: Total number of bacteria or cells, 5 million.

Note:

1. Reagent III, Reagent IV and sample should be placed on ice to avoid denaturation and inactivation.
2. The temperature of the reaction solution in the cuvette must be maintained at 37℃ or 25℃. Take a small beaker and add in a certain amount of distilled water and must keep the temperature be at 37℃ or 25℃. Place the beaker in a 37℃ or 25℃ water bath. In the reaction process, place the cuvette with the reaction solution in the beaker.
3. It is better for two people to do this experiment at the same time, one for measure the absorbance and one for timing to ensure the accuracy of the experimental results.
4. The activity of PFK in different homogenates is different. Please sit in 1-2 pre-experiments before the formal test. If ΔA>0.5, it means that the activity is too high. It is necessary to dilute homogenate supernatant to the appropriate concentration through using Extract solution (multiply the corresponding dilution times in the formula), or shorten the reaction time to 2 min or 5 min to make ΔA < 0.5, so as to improve the sensitivity of detection.

Experimental example:

1. 0.1 g of ryegrass is added into 1 mL of Extract solution for homogenization. After the supernatant is

taken out, the operation is carried out according to the determination steps. measured with a micro quartz cuvette: ΔA = A1-A2 =1.375-0.995=0.380.

PFK (U/g mass) = 535 × Δ A ÷ W = 450×0.380 ÷0.1=1710 U/g mass.

2. Take 0.1 g of peach leaves and add 1 mL of Extract solution for homogenization. After taking the supernatant, operate according to the determination steps. Measure with micro quartz cuvette and calculate ΔA=A1-A2 = 1.38-1.323=0.057.

PFK (U/g mass) = 535 × ΔA÷ W = 450×0.057 ÷0.1=256.5 U/g mass.

References：

[1] Papagianni M, Avramidis N. Lactococcus lactis as a cell factory: a twofold increase in phosphofructokinase activity results in a proportional increase in specific rates of glucose uptake and lactate formation[J]. Enzyme and microbial technology, 2011, 49(2): 197-202.

Related Products:

NA0714/NA0473 Pyruvate(PA) Content Assay Kit

NA0712/NA0471 Lactic Acid(LA) Content Assay Kit

NA0715/NA0474 Phosphoenolpyruvate Carboxylase(PEPC) Activity Assay Kit

NA0710/NA0469 Phosphoglycerate Kinase(PGK) Activity Assay Kit